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### Molecular imaging on the move

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# Chapter 5

## **Comparative biodistribution analysis across four different $^{89}\text{Zr}$ -monoclonal antibody tracers – The first step towards an imaging warehouse**

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## ABSTRACT

**Rationale:** Knowledge on monoclonal antibody biodistribution in healthy tissues in humans can support clinical drug development. Molecular imaging with positron emission tomography can yield information in this setting. However, recent imaging studies have analyzed the behavior of single antibodies only, neglecting comparison across different antibodies.

**Methods:** We compared the distribution of four  $^{89}\text{Zr}$ -labeled antibodies in healthy tissue in a retrospective analysis based on the recently published harmonization protocol for  $^{89}\text{Zr}$ -tracers and our delineation protocol.

**Results:** The biodistribution patterns of  $^{89}\text{Zr}$ -lumretuzumab,  $^{89}\text{Zr}$ -MMOT0530A,  $^{89}\text{Zr}$ -bevacizumab and  $^{89}\text{Zr}$ -trastuzumab on day 4 after tracer injection were largely similar. The highest tracer concentration was seen in healthy liver, spleen, kidney and intestines. About one-third of the injected tracer dose was found in the circulation, up to 15% in the liver and only 4% in the spleen and kidney. Lower tracer concentration was seen in bone marrow, lung, compact bone, muscle, fat and the brain. Despite low tracer accumulation per gram of tissue, large-volume tissues, especially fat, can influence overall distribution: On average, 5-7% of the injected tracer dose accumulated in fat, with a peak of 19% in a patient with morbid obesity.

**Conclusion:** The similar biodistribution of the four antibodies is probably based on their similar molecular structure, binding characteristics and similar metabolic pathways. These data provide a basis for a prospectively growing, online accessible warehouse of molecular imaging data, which enables researchers to increase and exchange knowledge on whole body drug distribution and potentially supports drug development decisions.

## INTRODUCTION

In the last two decades, 28 monoclonal antibodies (mAb), an important class of targeted anti-cancer therapeutics, have been approved by the FDA and EMA for cancer therapy.<sup>1-4</sup> The majority of these drugs were engineered to bind to a specific target. In addition, two antibody drug conjugates—mAbs loaded with a cytotoxic agent to specifically bring its cargo to cancer cells—are currently approved for use in patients. During the next decade much cancer drug development is expected to focus on antibodies or constructs based on antibodies, like antibody-drug conjugates, antibody fragments and bispecific antibodies.<sup>5</sup> As a result, rational drug dosing choices for mAbs are a crucial focus of clinical research, especially because the maximum tolerated doses often cannot be established.

This research can be enhanced with molecular imaging, which enables whole body assessment of drug distribution in healthy and malignant tissue over time. Moreover, molecular imaging can potentially enhance understanding of drug pharmacokinetics and support dosing decisions in early clinical drug development.<sup>6</sup> At present, nearly 20 mAbs have been coupled to various radionuclides and evaluated in early clinical trials using positron emission tomography (PET).<sup>6</sup> Of the radionuclides currently available, zirconium-89 ( $^{89}\text{Zr}$ ) is increasingly being used to label mAbs.  $^{89}\text{Zr}$  is advantageous because it remains in cells after internalization of the mAb-receptor complex, which leads to improved tumor image contrast via accumulation, and its half-life of about 78 hours allows target binding over a longer period of time, which better matches the long half-lives of mAbs.<sup>7</sup> A head-to-head comparison of Indium-111-trastuzumab and  $^{89}\text{Zr}$ -trastuzumab in mice showed the favorable image quality of the  $^{89}\text{Zr}$ -labeled tracer and a comparable normal organ distribution, with as the only difference a higher normal bone tracer uptake at the latest scan moment for  $^{89}\text{Zr}$ -trastuzumab.<sup>8</sup>

Until now, imaging trials only focused on biodistribution analysis of single agents, neglecting comparison across different antibodies. The latter, however, might be of great value during drug development, especially in the era of immune checkpoint inhibitors, as those target lymphoid (effector) tissue exclusively or in combination with tumor tissue potentially resulting in a different distribution pattern. Drug dose decisions might be optimized based on deeper knowledge of distribution, the influence of the size and/or structure of the molecule itself or the target. To discern specifics in the biodistribution of single mAbs, general knowledge about mAb distribution is required. Thereby, the administered protein dose is of special interest, as monoclonal antibodies can show different pharmacokinetic and potentially target saturation might occur after administration of therapeutic doses. Furthermore, as also engineered antibodies and other constructs like bispecific antibodies or antibody fragments with potentially different kinetics and dynamics are introduced in the clinics, more detailed information on distribution in combination with long term experience in dosing, side effects and efficacy of

the currently used mAb might speed up gain of knowledge.<sup>9</sup> Finally, molecular PET imaging with <sup>89</sup>Zr-mAb tracers seems to have the potential to predict response to mAb drug treatment.<sup>10</sup>

Previous multicenter trials with fluorine-18 have shown the necessity of harmonization to reduce data variability and enable comparison of data between trials and sites, which led to development of the European Association of Nuclear medicine (EANM) guidelines and establishment of the EANM Research Ltd (EARL) accreditation.<sup>11, 12</sup> For <sup>89</sup>Zr, a comparable harmonization protocol has been developed only recently.<sup>13</sup> Before this harmonization protocol became available, it was not possible to compare the biodistribution of <sup>89</sup>Zr-labeled mAbs assessed by PET. Comparison between the biodistribution of antibodies was also hampered by the lack of a standard delineation protocol when performing the semi-quantitative analyses. Moreover, biodistribution of some mAbs assessed in clinical imaging studies was described as part of a detailed dosimetric analysis (*e.g.*,<sup>14</sup>), whereas other imaging trials used a standardized uptake value (SUV) based description of the biodistribution (*e.g.*,<sup>15-18</sup>). If we could make standardized comparisons, this would be an important step towards using molecular imaging data for optimizing rational drug development.

To support such inter-study comparisons, we decided to generate the basis of a prospectively growing mAb imaging data warehouse. To do so, we performed a comparative biodistribution analysis of four <sup>89</sup>Zr-labeled mAbs that we previously explored in clinical studies: the anti-human epidermal growth factor receptor (HER) 2 antibody trastuzumab, the anti-HER3 antibody lumretuzumab, the anti-vascular endothelial growth factor A (VEGF-A) antibody bevacizumab and the anti-mesothelin antibody MMOT0530A.<sup>15-18</sup> This analysis was based on the <sup>89</sup>Zr-harmonization protocol and was conducted according to the delineation protocol for <sup>89</sup>Zr-tracers, which we recently established. We then deposited this data in an online warehouse.

## METHODS

### PET scan selection

For this analysis, we selected PET scans of patients following injection of the mAb tracers <sup>89</sup>Zr-lumretuzumab, <sup>89</sup>Zr-MMOT0530A, <sup>89</sup>Zr-bevacizumab or <sup>89</sup>Zr-trastuzumab. These scans were obtained from clinical imaging trials (specified below) or from extended clinical work-ups and had been performed before start of treatment on patients with locally advanced or metastatic solid cancer. The inclusion criteria for the scans were as follows: administered tracer activity of 37 MBq ( $\pm$  10%), the PET scan had to be performed 4 days after tracer injection together with a low-dose computed tomography (LD CT) and the tracer had to be complemented with the previously determined optimal unlabeled imaging protein dose (100 mg lumretuzumab, 50 mg trastuzumab, 10 mg MMOT0530A or no unlabeled mAb in case of bevacizumab imaging).<sup>17-19</sup> The

optimal unlabeled imaging protein dose was defined as the dose required to ensure sufficient tracer in the circulation 4 days after tracer injection for adequate tumor visualization. Patients and their corresponding PET scans were excluded from the biodistribution analysis if patient-related and/or disease-related factors were identified that might have influenced the biodistribution, like recurrent pleural effusion needing continuous drainage or a history of hemihepatectomy. Patients' history, age, sex, weight and height were collected from their dossiers.

PET scans were performed in clinical imaging trials executed in the Department of Medical Oncology of the University Medical Center Groningen (UMCG, Groningen, the Netherlands) and of the VU University Medical Center (VUMC, Amsterdam, the Netherlands),<sup>17, 18</sup> or as part of an extended clinical work-up. All clinical trials were approved by the responsible Medical Ethics Committee, as well as the Central Committee on Research Involving Human Subjects, and registered individually or as part of the corresponding phase I trial (ClinicalTrials.gov identifiers NCT01482377, NCT01832116 and NCT01028638).

## PET imaging

PET acquisition at 4 days postinjection was carried out from head to upper thigh, in bed positions of 5 minutes per bed position with a PET/CT camera (Biograph mCT, Siemens [UMCG], and Gemini TF or Ingenuity TF, Philips [VUMC]). PET scans were accompanied by a LD CT scan for attenuation correction. All PET images were reconstructed using the harmonized reconstruction algorithm recommended for multicenter <sup>89</sup>Zr-mAb PET scan trials.<sup>13</sup>

## <sup>89</sup>Zr-mAb PET scan analysis

PET scans of all four tracers were analyzed using the software A Medical Imaging Data Examiner (AMIDE version 0.9.1; Stanford University,<sup>20</sup>), in a standardized manner according to our delineation protocol (Supplementary Table 1). Spherical volumes of interest (VOI) with predefined sizes were drawn in healthy tissue in the brain, lung, liver, muscle, spleen, kidney, bone marrow, compact bone, fat and the intestine. A VOI in the aorta was used as readout for blood pool activity. Furthermore, all tumor lesions visible on PET and/or diagnostic CT scan were delineated per patient to estimate total tumor volume. In patients without liver metastases, the whole liver was manually delineated using the LD CT for anatomic reference to assess whole organ tracer uptake. A whole body VOI was drawn from head to the tuber ischiadicum to estimate the total amount of tracer left in the patient (excluding the legs).

For all VOIs, the percentage injected dose per kilogram (%ID/kg), normalized to calibrated dose of the <sup>89</sup>Zr-tracer, corrected for decay at the time of scanning, was calculated (Supplementary Table 1). The total tumor volume was estimated by the sum of single tumor volumes calculated based on the SUVmean40.<sup>21</sup> The percentage body fat<sup>22</sup> multiplied by patient's weight, and the

results of the delineation of the whole liver were used to calculate the activity as percentage of the injected dose (%ID) in total fat and liver, respectively (Supplementary Table 1). To assess the activity (%ID) in the spleen, kidneys and total blood volume, the volumes of the spleen and the kidneys were estimated based on linear CT measurements,<sup>23,24</sup> and the total blood volume was calculated using Nadler's formula.<sup>25</sup>

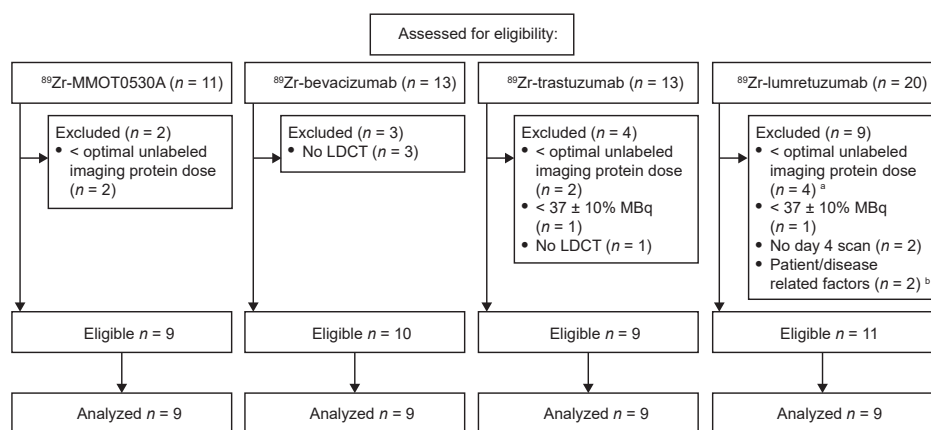
## Statistics

Statistical analyses were performed using IBM SPSS Version 22. In case of normally distributed data, groups were compared using a one-way ANOVA with either post hoc Gabriel or Games-Howell test depending on homogeneity of variances as assessed by Levene's test. If results were not normally distributed, comparison was performed using a Kruskal-Wallis followed by a Mann-Whitney U test.  $P \leq 0.05$  was considered to be a significant difference. All analyses were 2-sided. Bivariate correlations were performed using Pearson correlation coefficients. Data are presented as mean  $\pm$  standard deviation (SD), unless otherwise stated.

## RESULTS

### Patient characteristics

For  $^{89}\text{Zr}$ -lumretuzumab,  $^{89}\text{Zr}$ -MMOT0530A,  $^{89}\text{Zr}$ -bevacizumab and  $^{89}\text{Zr}$ -trastuzumab, scans of eleven, nine, ten and nine patients, respectively, were eligible for comparative  $^{89}\text{Zr}$ -tracer biodistribution analysis (Fig. 1). Per tracer, we analyzed the same number of scans ( $n = 9$ ). If



**Figure 1** Scan selection per tracer according to predefined eligibility criteria. <sup>a</sup> Optimal imaging dose of unlabeled lumretuzumab was considered 100 mg, scans with 10 and 50 mg were excluded. <sup>b</sup> The scans of patients with a history of a hemihepatectomy ( $n = 1$ ) and recurrent pleural effusion needing continuous drainage ( $n = 1$ ) were excluded.

more than 9 scans were available, the first 9 of all eligible PET scans were selected. Additional information on the mAbs and the respective <sup>89</sup>Zr-tracers are summarized in Supplementary Table 2. The analyzed PET scans were performed between November 2009 and August 2014. Included patients had locally advanced or metastatic cancer, the majority of the patients had multiple metastatic sites. Patient characteristics are summarized in Table 1.

**Table 1 Patient characteristics**

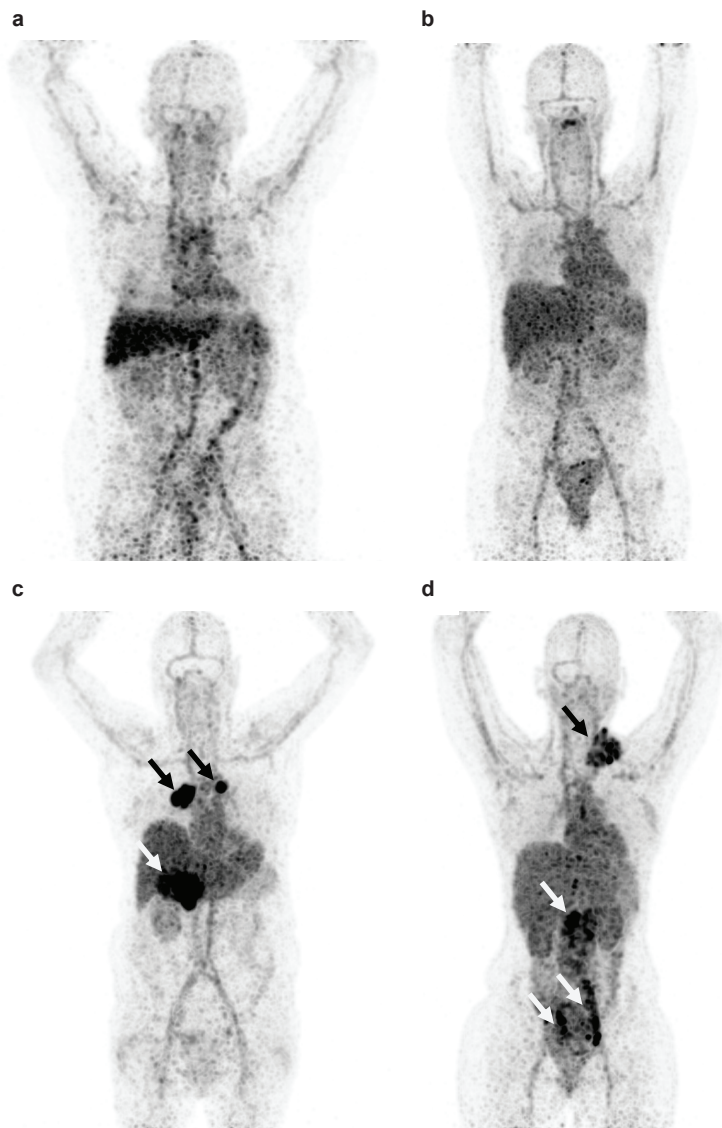
Characteristics	<sup>89</sup> Zr-lumretuzumab <sup>a</sup>	<sup>89</sup> Zr-MMOT0530A	<sup>89</sup> Zr-bevacizumab	<sup>89</sup> Zr-trastuzumab <sup>b</sup>
Age, median years (range)	59 (48-72)	62 (44-70)	64 (51-68)	47 (22-73)
Number of patients	9	9	9	9
Male	5 (56)	2 (22)	5 (56)	2 (22)
Female	4 (44)	7 (78)	4 (44)	7 (78)
Tumor type, <i>n</i> (%)				
Colorectal	5 (56)	-	-	1 (11)
Breast	1 (11)	-	-	8 (89)
Oropharyngeal	1 (11)	-	-	-
CUP	1 (11)	-	-	-
Vulvar	1 (11)	-	-	-
Ovarian	-	2 (22)	-	-
Pancreatic	-	7 (78)	-	-
Renal cell	-	-	9 (100)	-
Sites of tumor lesions, <i>n</i> <sup>c</sup>				
Adrenal gland	3	3	3	-
Bone	1	-	40	121
Brain	-	-	2	9
Breast	-	-	-	2
Intestine	2	-	2	1
Kidney	-	-	16	-
Lung	34	3	6	2
Liver	-	7	7	48
Lymph nodes	10	6	9	27
Pancreas	-	4	4	-
Pleura	2	-	3	-
Soft tissue <sup>d</sup>	10	6	29	6
Spleen	-	-	1	1
Thyroid gland	-	-	2	-
Uterus	-	2	-	-
Tumor load, mL (± SD)	41 (± 25)	42 (± 22)	116 (± 67)	99 (± 133)

<sup>a</sup> <sup>89</sup>Zr-lumretuzumab PET was performed in patients with HER3-positive disease according to study protocol (Meulendijks et al., CCR 2017). <sup>b</sup> <sup>89</sup>Zr-trastuzumab PET was performed in patients with HER2-positive disease as part of an extended clinical work-up. <sup>c</sup> Sites of tumor lesions comprise sites of primary tumors and sites of metastases at the time of PET imaging. <sup>d</sup> Soft tissue lesions include subcutaneous lesions not further specified, intramuscular lesions and abdominal soft tissue lesions. CUP, cancer of unknown primary.



### **$^{89}\text{Zr}$ -mAb tracer distribution in healthy tissue**

All tracers showed a comparable distribution pattern throughout the whole body with the highest tracer uptake (calculated as %ID/kg) in healthy liver tissue, as well as in the spleen, kidneys and intestines (Fig. 2). Generally, lower tracer concentration was observed in the bone marrow, lung, compact bone, muscle, fat tissue and the brain (Table 2).



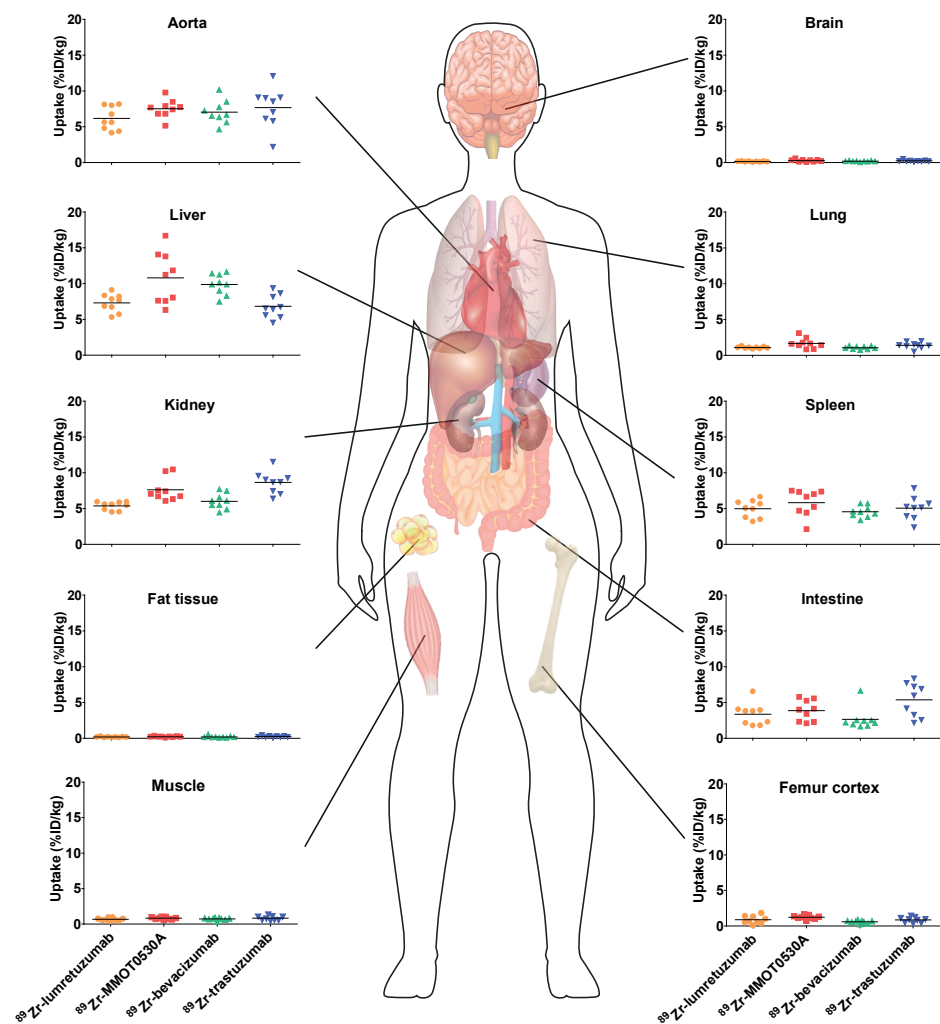
**Figure 2** Representative maximum intensity projection of a PET scan 4 days after injection with  $^{89}\text{Zr}$ -lumretuzumab (a),  $^{89}\text{Zr}$ -MMOT0530A (b),  $^{89}\text{Zr}$ -bevacizumab (c) and  $^{89}\text{Zr}$ -trastuzumab (d). The patients injected with  $^{89}\text{Zr}$ -bevacizumab (c) and  $^{89}\text{Zr}$ -trastuzumab (d) show clear tumor tracer uptake (black and white arrows), whereas tumor lesions on the  $^{89}\text{Zr}$ -lumretuzumab PET and  $^{89}\text{Zr}$ -MMOT0530A were visually negative.

**Table 2** Normal tissue tracer uptake as percent of injected dose per kilogram bodyweight per <sup>89</sup>Zr-monoclonal antibody tracer 4 days post tracer injection

Tissue	VOI (%ID/kg, ± SD)				ANOVA/ Kruskal-Wallis <i>P</i>
	<sup>89</sup> Zr-lumretuzumab	<sup>89</sup> Zr-MMOT0530A	<sup>89</sup> Zr-bevacizumab	<sup>89</sup> Zr-trastuzumab	
Aorta	6.2 (± 1.6)	7.5 (± 1.3)	7.1 (± 1.6)	7.7 (± 2.8)	0.35
Bone marrow	1.9 (± 0.6)	2.9 (± 0.5)	2.1 (± 0.7)	2.8 (± 1.0)	<b>0.02</b> <sup>a</sup>
Brain	0.1 (± 0.1)	0.3 (± 0.2)	0.2 (± 0.1)	0.3 (± 0.1)	0.09
Compact bone	0.9 (± 0.6)	1.2 (± 0.3)	0.6 (± 0.2)	0.8 (± 0.4)	<b>0.01</b> <sup>b</sup>
Fat tissue	0.2 (± 0.1)	0.2 (± 0.1)	0.2 (± 0.2)	0.3 (± 0.1)	0.26
Intestine	3.4 (± 1.5)	3.9 (± 1.5)	2.7 (± 1.5)	5.4 (± 2.4)	<b>0.02</b> <sup>c</sup>
Kidney	5.4 (± 0.6)	7.6 (± 1.6)	6.1 (± 1.1)	8.7 (± 1.5)	<b>&lt; 0.01</b> <sup>d</sup>
Liver	7.3 (± 1.3)	10.8 (± 3.6)	9.9 (± 1.4)	6.8 (± 1.6)	<b>&lt; 0.01</b> <sup>e</sup>
Lung	1.1 (± 0.1)	1.7 (± 0.7)	1.1 (± 0.2)	1.4 (± 0.4)	<b>0.02</b> <sup>a</sup>
Muscle	0.7 (± 0.2)	0.8 (± 0.2)	0.7 (± 0.2)	0.8 (± 0.3)	0.36
Spleen	5.0 (± 1.2)	5.8 (± 1.8)	4.6 (± 0.8)	5.1 (± 1.6)	0.32

<sup>a</sup> Post hoc analysis showed no significant difference between the four groups. <sup>b</sup> Significant difference between <sup>89</sup>Zr-MMOT0530A and <sup>89</sup>Zr-bevacizumab. <sup>c</sup> Significant difference between <sup>89</sup>Zr-bevacizumab and <sup>89</sup>Zr-trastuzumab. <sup>d</sup> Significant difference between <sup>89</sup>Zr-lumretuzumab and <sup>89</sup>Zr-MMOT0530A, <sup>89</sup>Zr-lumretuzumab and <sup>89</sup>Zr-trastuzumab, <sup>89</sup>Zr-bevacizumab and <sup>89</sup>Zr-trastuzumab, and <sup>89</sup>Zr-MMOT0530A. <sup>e</sup> Significant difference between <sup>89</sup>Zr-lumretuzumab and <sup>89</sup>Zr-bevacizumab, <sup>89</sup>Zr-bevacizumab and <sup>89</sup>Zr-trastuzumab, and <sup>89</sup>Zr-trastuzumab and <sup>89</sup>Zr-MMOT0530A. VOI, volume of interest.

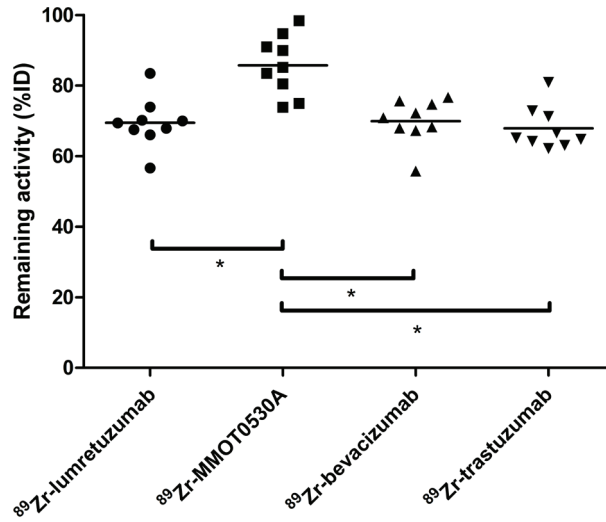
Healthy lung tracer uptake was the highest for <sup>89</sup>Zr-MMOT0530A compared to the other tracers with a mean of 1.7 (± 0.7) %ID/kg (Fig. 3 and Table 2). Furthermore, lung uptake varied most between patients injected with <sup>89</sup>Zr-MMOT0530A (range 0.8-3.1 vs. 0.9-1.3, 0.7-1.4 and 0.5-2.0 for patients injected with <sup>89</sup>Zr-lumretuzumab, <sup>89</sup>Zr-bevacizumab and <sup>89</sup>Zr-trastuzumab, respectively). Also uptake in healthy liver tissue, compact bone and bone marrow was the highest for <sup>89</sup>Zr-MMOT0530A (10.8 ± 3.6, 1.2 ± 0.3 and 2.9 ± 0.5 %ID/kg). In the intestine, likely influenced by fecal content, as well as in healthy renal tissue, the highest activity, representing at least partially excretion, was observed for <sup>89</sup>Zr-trastuzumab (5.4 ± 2.4 and 8.7 ± 1.5 %ID/kg). For brain, spleen, muscle and fat tissue comparable uptake was observed between the four analyzed <sup>89</sup>Zr-mAb tracers. The remaining radioactivity present in the aorta (= readout for blood pool) was similar between all <sup>89</sup>Zr-mAb tracers as a result of the added unlabeled antibody imaging dose selected in earlier trials. Blood pool activity did not correlate with the tumor load for <sup>89</sup>Zr-lumretuzumab, <sup>89</sup>Zr-bevacizumab and <sup>89</sup>Zr-MMOT0530A, and only poorly for <sup>89</sup>Zr-trastuzumab ( $r^2 = 0.46$ , Supplementary Fig. 1).



**Figure 3** Tracer uptake (%ID/kg) per healthy tissue and in blood for  $^{89}\text{Zr}$ -lumretuzumab,  $^{89}\text{Zr}$ -MMOT0530A,  $^{89}\text{Zr}$ -bevacizumab and  $^{89}\text{Zr}$ -trastuzumab (left to right) 4 days post tracer injection. Each dot represents an individual patient; mean is plotted per tracer.

### Amount of tracer in healthy tissue, as percentage of injected dose

There was no difference between the total amount of  $^{89}\text{Zr}$ -lumretuzumab,  $^{89}\text{Zr}$ -bevacizumab and  $^{89}\text{Zr}$ -trastuzumab remaining in the body on day 4 after tracer injection ( $69.5 \pm 7.1$ ,  $69.9 \pm 6.3$  and  $67.9 \pm 6.1$  %ID, respectively). The remaining activity in patients injected with  $^{89}\text{Zr}$ -MMOT0530A was higher compared to the other three tracers ( $85.8 \pm 8.5$  %ID) (Fig. 4).



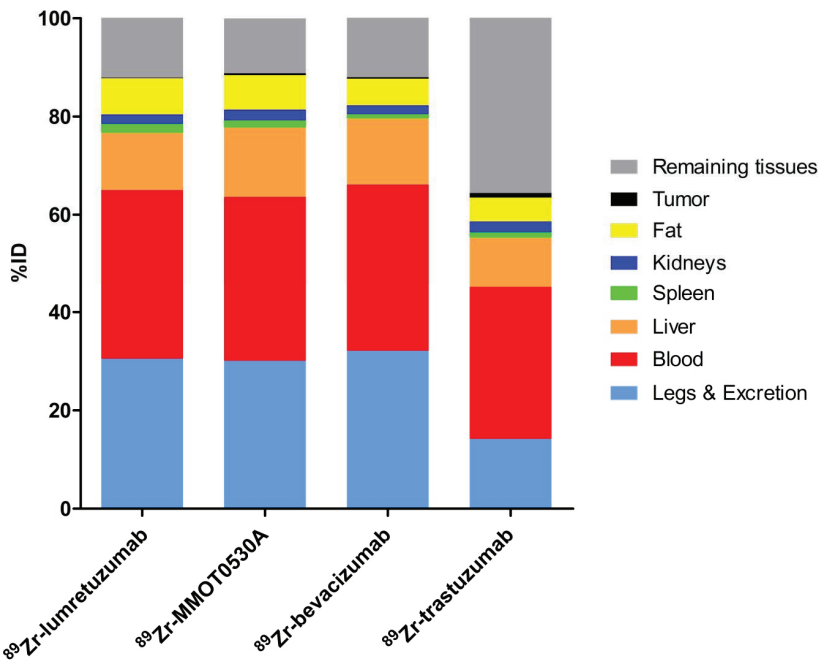
**Figure 4** Radioactivity as %ID left in the body (head to tuber ischiadicum) 4 days postinjection per tracer. Each dot represents individual patients and asterisk indicates differences between groups with  $P < 0.05$ .

A mean of  $34.5 (\pm 8.4)$ ,  $33.5 (\pm 3.7)$ ,  $34.0 (\pm 5.7)$  and  $30.9 (\pm 12.5)$  %ID was still circulating in the blood 4 days after administration of  $^{89}\text{Zr}$ -lumretuzumab,  $^{89}\text{Zr}$ -MMOT0530A,  $^{89}\text{Zr}$ -bevacizumab and  $^{89}\text{Zr}$ -trastuzumab, respectively (Table 3 and Fig. 5). In patients without liver metastases on average  $10.3 (\pm 1.2, ^{89}\text{Zr}$ -trastuzumab) %ID up to  $14.0 (\pm 0.7, ^{89}\text{Zr}$ -MMOT0530A) %ID accumulated in the liver 4 days post injection ( $n = 5, 3, 4$  and  $3$  patients who received, respectively,  $^{89}\text{Zr}$ -lumretuzumab,  $^{89}\text{Zr}$ -MMOT0530A,  $^{89}\text{Zr}$ -bevacizumab and  $^{89}\text{Zr}$ -trastuzumab). The total liver uptake ranged from  $8.5$  %ID to  $16.0$  %ID (Fig. 6). Despite the relatively high tracer concentration in the

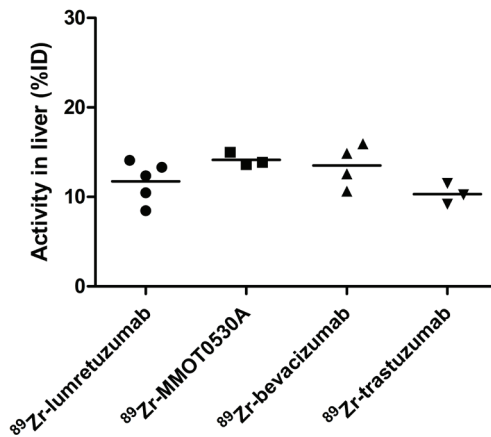
**Table 3** Activity as part of the injected dose per  $^{89}\text{Zr}$ -monoclonal antibody tracer 4 days post tracer injection

Tissue	VOI (%ID, $\pm$ SD)			
	$^{89}\text{Zr}$ -lumretuzumab	$^{89}\text{Zr}$ -MMOT0530A	$^{89}\text{Zr}$ -bevacizumab	$^{89}\text{Zr}$ -trastuzumab
Total blood pool	$34.5 (\pm 8.4)$	$33.5 (\pm 3.7)$	$34.0 (\pm 5.7)$	$30.9 (\pm 12.5)$
Liver	$11.7 (\pm 2.3)$	$14.2 (\pm 0.7)$	$13.5 (\pm 2.4)$	$10.3 (\pm 1.2)$
Spleen	$1.8 (\pm 1.0)$	$1.4 (\pm 0.5)$	$0.9 (\pm 0.2)$	$1.0 (\pm 0.1)$
Kidney	$1.0 (\pm 0.4)$	$1.1 (\pm 0.3)$	$1.6 (\pm 0.9)$	$1.1 (\pm 0.1)$
Fat tissue	$7.4 (\pm 5.1)$	$7.0 (\pm 2.2)$	$5.4 (\pm 2.4)$	$4.9 (\pm 2.7)$
Tumor	$0.1 (\pm 0.1)$	$0.4 (\pm 0.3)$	$0.3 (\pm 0.2)$	$0.9 (\pm 1.8)$

VOI, volume of interest.

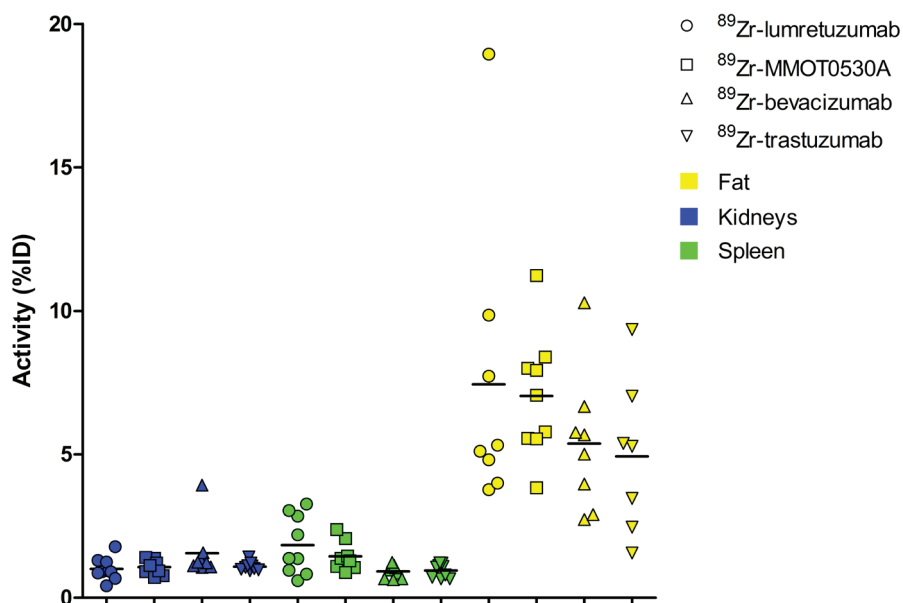


**Figure 5** Mean uptake as %ID per  $^{89}\text{Zr}$ -monoclonal antibody tracer in blood, liver, spleen, kidney, fat, tumor, remaining tissues (including intestines), as well as excreted part and the legs.



**Figure 6** Radioactivity as %ID in the liver without metastatic disease could be assessed in 5, 3, 4 and 3 patients injected with  $^{89}\text{Zr}$ -lumretuzumab,  $^{89}\text{Zr}$ -MMOT0530A,  $^{89}\text{Zr}$ -bevacizumab and  $^{89}\text{Zr}$ -trastuzumab, respectively.

spleen (up to  $5.8 \pm 1.8$  %ID/kg) and kidney (up to  $8.7 \pm 1.5$  %ID/kg), the absolute proportion of tracer accumulating in these organs was only 0.6 to 3.3 %ID for the spleen and 0.4 to 3.9 %ID for one kidney (Fig. 7). Tracer concentration in fat tissue, in contrast, was low compared to other healthy tissues:  $0.2 (\pm 0.1)$ ,  $0.2 (\pm 0.1)$ ,  $0.2 (\pm 0.2)$  and  $0.3 (\pm 0.1)$  %ID/kg for  $^{89}\text{Zr}$ -lumretuzumab,



**Figure 7** Radioactivity as %ID per  $^{89}\text{Zr}$ -monoclonal antibody tracer in kidney, spleen and fat per patient per tracer (mean).

$^{89}\text{Zr}$ -MMOT0530A,  $^{89}\text{Zr}$ -bevacizumab and  $^{89}\text{Zr}$ -trastuzumab, respectively. Despite this generally low tracer concentration in fat, when the total amount of fat tissue in the patient was considered, a substantial proportion of the injected tracer dose accumulated in fat tissue:  $7.4 (\pm 5.4)$ ,  $7.0 (\pm 2.2)$ ,  $5.4 (\pm 2.6)$  and  $4.9 (\pm 2.8)$  %ID of  $^{89}\text{Zr}$ -lumretuzumab,  $^{89}\text{Zr}$ -MMOT0530A,  $^{89}\text{Zr}$ -bevacizumab and  $^{89}\text{Zr}$ -trastuzumab, respectively; Fig. 5. The estimated total amount of tracer accumulated in fat tissue ranged between 1.56 %ID and 18.95 %ID depending on the physique of the patient (Fig. 7).

The median measurable tumor load was  $41 (\pm 25)$  mL,  $42 (\pm 22)$  mL,  $116 (\pm 67)$  mL and  $99 (\pm 133)$  mL for  $^{89}\text{Zr}$ -lumretuzumab,  $^{89}\text{Zr}$ -MMOT0530A,  $^{89}\text{Zr}$ -bevacizumab and  $^{89}\text{Zr}$ -trastuzumab, respectively (Table 1). In this dataset, on average per tracer less than 1 %ID accumulated in tumor lesions per patient (Table 3).

## DISCUSSION

We conducted the first comparative analysis of the healthy tissue distribution of four  $^{89}\text{Zr}$ -mAb tracers, based on the recently published harmonization protocol for  $^{89}\text{Zr}$  <sup>13</sup> and analyzed according to our delineation protocol for  $^{89}\text{Zr}$ -mAb tracers.

$^{89}\text{Zr}$ -lumretuzumab,  $^{89}\text{Zr}$ -MMOT0530A,  $^{89}\text{Zr}$ -bevacizumab and  $^{89}\text{Zr}$ -trastuzumab showed a similar healthy tissue distribution pattern, with highest uptake in the liver, spleen and kidneys

and lower uptake in the bone marrow, lung, compact bone, muscle, fat tissue and the brain. Tissues with generally low uptake can also be of interest with regards to distribution if the respective tissue comprises a larger volume, potentially influencing (optimal) tumor targeting. Fat tissue, for example, had one of the lowest uptake values in our dataset. However, depending on the physique of the patient, up to 19 %ID accumulated in fat. In comparison, the liver, which often is considered as sink organ by visual analysis due to its high tracer uptake per kg tissue, contained about 10-15 %ID and the spleen and one kidney account for a maximum of 4 %ID each, whereas less than 1 %ID accumulated in tumor lesions. The observed slight differences in healthy tissue distribution between the four analyzed  $^{89}\text{Zr}$ -mAb tracers might be the consequence of target specific uptake, non-specific tracer accumulation and/or clearance mechanisms specific for the certain mAb.

$^{89}\text{Zr}$ -MMT0530A uptake, compared to the other three tracers, was higher in the lung, which might partly be explained by the expression of mesothelin in mesothelial cells *e.g.* in the lining of the pleura,<sup>26,27</sup> whereas higher uptake in the liver, compact bone and bone marrow cannot be explained by the known protein expression profile. HER2 expression in and tracer excretion via the intestinal and urinary tract, in contrast, might have influenced  $^{89}\text{Zr}$ -trastuzumab uptake in the respective healthy tissues.<sup>28</sup> Different from studies on  $^{111}\text{In}$ -trastuzumab performed shortly after cardiotoxic anthracycline treatment, in the present study cardiac HER2 expression was not visualized by  $^{89}\text{Zr}$ -trastuzumab PET. This was probably due to a receptor expression level that was below the PET-detectable threshold in the unstressed heart of the imaged patient population.<sup>29,30</sup>

The location of the target itself, either in the cell membrane or in the surroundings of the tumor, did not seem to have much influence on the healthy tissue distribution of the four analyzed tracers;  $^{89}\text{Zr}$ -bevacizumab, which targets a non-membrane-bound growth factor, showed the same pattern as the other three mAbs that bind to membranous receptors. Besides tumor localized target, circulating target *e.g.*, as soluble receptor or expressed by circulating tumor cells could also influence tracer kinetics. For all four targets, circulating variants have been described, but the impact of this fraction on drug kinetics, however, is unclear.<sup>31-34</sup> Next to the ability of the analyzed mAbs to internalize in tumor cells, which was also indicated for bevacizumab,<sup>35</sup> the absolute amount of target expressed, which is largely influenced by overall tumor load, might affect mAb tracer kinetics. In our dataset, however, the proportion of tracer localized at tumor sites was quite low, probably due to the low mean tumor load of the selected patients. At least for trastuzumab, it has been shown previously that tumor load clearly influences tracer and drug kinetics.<sup>36</sup>

Tracer distribution in healthy organs might also be influenced by non-target specific factors, such as the amount of tracer available in the blood, as well as metabolism and excretion.<sup>37,38</sup> Together with the  $^{89}\text{Zr}$ -labeled mAb, a dose of unlabeled protein, which had been determined

in the previous imaging trials with the respective mAb, was injected. The unlabeled protein dose increases tracer availability over time, which is necessary with mAbs with dose-dependent kinetics like trastuzumab.<sup>36,39</sup> Per tracer, the optimal unlabeled protein dose for baseline imaging was selected to assure comparable activity concentration in the blood of all four tracers over time. However, with all analyzed tracers apart from  $^{89}\text{Zr}$ -MMOT0530A, PET imaging has also been performed after administration of pharmacodynamics-active doses or the actual therapeutic dose.<sup>15, 16, 40</sup> Thereby, normal organ distribution was comparable between baseline imaging and on-treatment imaging, whereas tumor uptake changed over time. Next to the unlabeled protein dose, the amount of endogenous neonatal Fc receptor (FcRn) might also influence the circulation time of therapeutic immune globulin G (IgG) molecules by protecting them from fast degradation, resulting in a long half-life of more than 20 days.<sup>41,42</sup> All four mAbs are IgG1 molecules, so binding specifications to the FcRn are expected to be comparable, potentially explaining the similar biodistribution (Supplementary Table 2).

Due to their size, monoclonal antibodies like the ones studied here, are metabolized first to peptides and amino acids before being re-used for protein synthesis or excreted via the kidneys.<sup>42</sup> Several target and non-target specific mechanisms, as well as proteolysis by the liver and the reticuloendothelial system, contribute to mAb elimination. Advanced mAb engineering such as glycosylation can increase uptake and metabolism of mAbs by hepatic non-parenchymal Kupffer cells located in the liver, which might explain the relatively higher healthy liver tracer uptake.<sup>42,43</sup> After elimination, non-used remnants like the radionuclide can be excreted via the intestinal tract or potentially accumulate in compact bone or bone marrow, as visualized by PET. Furthermore, differences in tracer intactness over time might explain the differing uptake in compact bone.

The scientific community is becoming increasingly aware of the many benefits of data sharing.<sup>44</sup> An example of the usefulness of data sharing is provided by the RECIST criteria, which after development have been verified by a warehouse containing data from numerous trials.<sup>45</sup> With this first comparative analysis of four  $^{89}\text{Zr}$ -labeled mAb we aimed to create a basis for a prospectively growing warehouse of molecular imaging data of antibody-based tracers that will enable researchers worldwide to enhance and share their knowledge on whole body drug distribution. Therefore, all researchers in the field of molecular imaging are encouraged to add data to this developing warehouse as only expansion of the available data is expected to deliver a valuable long-term gain of knowledge. A short description on how to share or request imaging data are provided in Supplementary Table 3. We aim to add data from ongoing and planned imaging trials assessing molecules belonging to another IgG subclass, with different molecule size or structure, will be added to this warehouse in the future. Potentially target saturation can occur with higher antibody doses than used for baseline imaging and changes in biodistribution might occur based on repeated therapeutic dosing. Therefore, addition and



comparison of biodistribution of the same antibody with varying unlabeled antibody doses for imaging will be of interest as well.

### **Abbreviations**

AMIDE: A medical imaging data examiner; (LD) CT: (Low dose) computed tomography; CUP: Cancer of unknown primary; EANM: European Association of Nuclear Medicine; EARL: EANM Research Ltd; FcRn: Neonatal Fc receptor; HER: Human epidermal growth factor receptor; ID: Injected dose; IgG: Immunoglobulin G; mAb: Monoclonal antibody; PET: Positron emission tomography; SD: Standard deviation; VEGF-A: Vascular endothelial growth factor A; VOI: Volume of interest; <sup>89</sup>Zr: Zirconium-89.

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### **Competing interests**

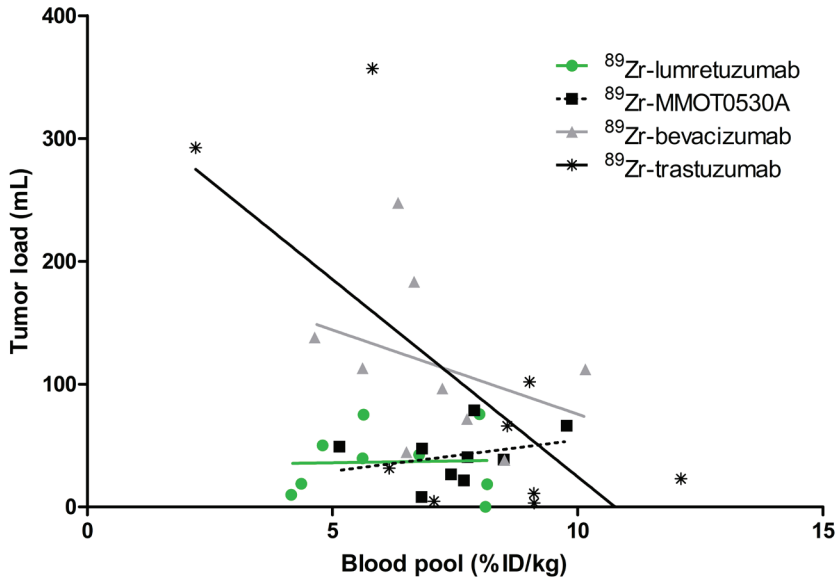
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## SUPPLEMENTARY MATERIALS



**Supplementary Figure 1** Correlation between activity in the aorta (%ID/kg) and tumor load (mL) for  $^{89}\text{Zr}$ -lumretuzumab ( $r^2 = 0.00$ ,  $P = 0.93$ ),  $^{89}\text{Zr}$ -MMOT0530A ( $r^2 = 0.09$ ,  $P = 0.44$ ),  $^{89}\text{Zr}$ -bevacizumab ( $r^2 = 0.11$ ,  $P = 0.38$ ) and  $^{89}\text{Zr}$ -trastuzumab ( $r^2 = 0.46$ ,  $P = 0.05$ ).

## Supplementary Table 1 Standard delineation protocol

### **1. Reconstruction method**

According to Makris et al.<sup>1</sup>

### **2. Analysis plan**

PET image analysis of scans performed 4 days post tracer injection.

Targets to select for PET quantification

- Tumor lesions
- Background regions (=healthy organs/tissue)
- Whole organs

PET quantification parameters

- Background regions: %ID/kg
- Tumor lesions: volume (mL)
- Whole organs: %ID/kg

Software

- A medical imaging data examiner (AMIDE, <sup>2</sup>)

### **Targets**

Lesions

- All visible lesions on PET and/or on diagnostic CT scan

Background regions

- For each background organ a background area should be quantified.
- Use an sphericalVOI (location and/or size of VOI might be adapted in case of tumor locations) in at least 3 consecutive axial planes:
  - Brain 5 cm (left hemisphere, parietal)
  - Lung 5 cm (right upper lobe, mediolateral)
  - Aortic blood pool 2 cm (Aortic arch or thoracic aorta, highest region)
  - Muscle 5 cm (region right gluteus maximus/medius)
  - Spleen 5 cm (representative region; 4 cm if 5 cm VOI is too big) and 2 cm (highest region)
  - Liver 5 cm (representative region)
  - Kidney 2 cm (cortex of left kidney, highest region)
  - Bone marrow 2 cm (L4 or L5)
  - Bone cortex 1 cm (femur cortex, right)

- Intestine 2 cm (highest region)
- Fat tissue 2 cm (abdominal region)

Whole organ analysis

- Only assess organs when there is no metastatic disease located in this certain organ
- Organs of interest for whole organ analysis:
  - Liver

### Calculations

AMIDE output (mean activity concentration in Bq/cc) was used to calculate the percentage injected dose per kilogram (%ID/kg) tissue of every VOI with the following formula:

$$\%ID/kg = \frac{\text{Activity concentration (Bq/kg)}}{\text{Injected activity (Bq)}} * 100\%$$

Injected activity was corrected for decay between moment of tracer injection and time of scanning (under the assumption of a tissue density of 1 kg/L).

Percentage organ and fat tissue tracer uptake was calculated using the following formula:

$$\text{Organ uptake (\%)} = \frac{\text{Activity concentration (Bq/gr)} * \text{Organ volume (gr)}}{\text{Injected activity (Bq)}} * 100\%$$

**Supplementary Table 2 Detailed information antibody and tracer characteristics**

	<sup>89</sup> Zr-lumretuzumab	<sup>89</sup> Zr-MMOT0530A	<sup>89</sup> Zr-bevacizumab	<sup>89</sup> Zr-trastuzumab
Pharmacokinetic parameters of monoclonal antibody				
Monoclonal antibody	Lumretuzumab	MMOT0530A	Bevacizumab	Trastuzumab
IgG class	Humanized glycoengineered IgG1κ	Humanized IgG1	Humanized IgG1	Humanized IgG1
Target	HER3	Mesothelin	VEGF	HER2
Molecular weight (kDa)	150	150	150	150
Linear kinetics	Elimination of lumretuzumab across dose range 100-400 mg is predominantly target mediated; PK approached linearity at 400-2000 mg	Modest degree of target mediated clearance at doses < 1 mg/kg; linear clearance across tested dose range of 0.2 to 2.8 mg/kg for the q3w schedule	Linear pharmacokinetics for doses 1-10 mg/kg	Non-linear elimination
Clearance	1.04 L/d (100 mg); 0.264 L/d (> 2000 mg)	27 mL/d/kg	0.188 L/d - 0.220 L/d	0.111 L/d
Volume of distribution	3.64 L (100 mg); 4.4 L (> 2000 mg)	V <sub>ss</sub> = 68 mL/kg	2.73-3.28 L	2.91 L
Elimination half-life time	2.4 d (100 mg); 12 d (> 400 mg)	2.1-3.7 d	18-20 d	28.5 d
Reference	Meulendijks et al. <sup>3</sup>	Weekes et al. <sup>4</sup>	European public assessment report of Herceptin <sup>5</sup>	European public assessment report of Avastin <sup>6</sup>

Information on the <sup>89</sup> Zr-labeled antibodies				
Chelator	TFP-N-sucDf	TFP-N-sucDf	TFP-N-sucDf	TFP-N-sucDf
Chelator:mAb conjugation ratio	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.3 ± 0.1
In vitro serum stability	Stable in serum; < 5% decrease in radiochemical purity (rcp) after 168 h	2% decrease in rcp after 168 h in normal saline at 20°C	6% decrease rcp after 168 h in serum	0.39 ± 0.02% decrease in rcp/day in serum
Radiochemical purity (%)	> 98	> 98	> 98	> 98
pH	4-7	5-8	6-7	5-8
Immunoreactivity (%)	Preserved	> 70	> 60	> 70
Appearance	Colorless to light yellow	Colorless to light yellow liquid	Colorless	Colorless
Bacterial endotoxins (EU/mL)	< 2.5	< 2.5	< 1.0	< 2
Aggregates (%)	< 5	< 5	< 3	< 5
Sterility	Sterile	Sterile	Sterile	Sterile

<sup>89</sup>Zr, zirconium-89; IgG, immunoglobuline gamma; HER, human epidermal growth factor receptor; mAb, monoclonal antibody; rcp, radiochemical purity; TFP-N-sucDf, tetrafluorophenol-N-succinyl-desferal; VEGF, vascular endothelial growth factor receptor; Vss, steady state volume of distribution.



## Supplementary Table 3 Details on deposited data and curation process

### **Data deposit**

An overview over the deposited datasets including details on the dataset, contact information, information on requesting and depositing data can be found online under [www.imagingwarehouse.eu](http://www.imagingwarehouse.eu).

### **Deposited data**

Information on the individual subject and imaging data per individual subject will be deposited.

Specification of the deposited data for the four  $^{89}\text{Zr}$ -mAb tracers analyzed in the current manuscript:

#### **Patient related information:**

Weight, height, total tumor load (PET based, mL), injected [netto] dose, time between tracer injection and start of PET scan, activity on the day of tracer injection.

#### **PET imaging data per individual patient:**

AMIDE output and SUV calculations for blood and normal organ VOI's: aorta, liver, kidney, fat tissue, muscle, brain, lung, spleen, intestine, femur cortex and bone marrow.

AMIDE output per VOI includes median, mean, variance, standard deviation, minimum, maximum and size ( $\text{mm}^3$ ).

SUV calculations include SUVmean and SUVmax.

Data to be deposited by external parties should include at least above mentioned patient related information and PET related information. Thereby, the administered radiation dose is not restricted to 37 MBq, as the dose can vary. Information on the used analysis tool and/or algorithm should also be deposited. Throughout time, requirements on which data to be deposited might change, therefore, it is recommended to consult the website for further instructions ([www.imagingwarehouse.eu](http://www.imagingwarehouse.eu)).

### **Data request**

All data will be provided upon request. Requests can be send by email to the imaging warehouse group ([imagingwarehouse@onco.umcg.nl](mailto:imagingwarehouse@onco.umcg.nl)). Data can be requested by health care professionals and all scientific personnel. Data is provided for research purpose only.

Re-processing of imaging data with other reconstruction protocols and additional information can be requested. Whether requested data can be provided, will be decided by the for the dataset responsible researcher or delegates (e.g. based on privacy laws).

**Request format**

All requests need to contain a specification of the requested data set, information on the requesting person or group including name of the responsible investigator, function and institution. Furthermore, a short description of the intended aim/research question is preferred.

**Public disclosure and publication policy**

Provenance of the data must be stated and data needs to be referenced to in all publications in written form, oral presentation or publication in any other form.

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